

What is claimed is:

1. A cell composition comprising a population of non-yeast eukaryotic cells containing a diverse population of about 10 or more variant nucleic acids, each of said variant nucleic acids being expressed in a different cell and located within each cell at an identical site in the genome.
2. The cell composition of claim 1, wherein said variant nucleic acids have predetermined amino acid changes at preselected positions within a parent amino acid sequence.
3. The cell composition of claim 1, wherein said variant nucleic acids are integrated in each cell by a site specific recombination sequence.
4. The cell composition of claim 1, wherein said cells express Cre recombinase or Flp recombinase.
5. The cell composition of claim 1, wherein said site in the genome comprises two lox sites.
6. The cell composition of claim 5, wherein at least one of said lox sites is a loxP site.
7. The cell composition of claim 5, wherein at least one of said lox sites is a lox511 site.
8. The cell composition of claim 5, wherein said site in the genome comprises two non-identical lox sites.

502/550

SUB
A8

9. The cell composition of claim 8, wherein said site in the genome comprises a loxP site and a lox511 site.

10. The cell composition of claim 1, wherein
5 said cell is a mammalian cell.

11. A method of identifying a polypeptide exhibiting optimized activity, comprising:

(a) screening the cell composition of claim 1 for an activity associated with a parent polypeptide of a
10 diverse population of variant polypeptides encoded by said variant nucleic acids; and

(b) identifying a variant polypeptide exhibiting an optimized activity relative to said parent polypeptide.

15 12. A method of identifying a binding ligand, comprising:

(a) contacting the cell composition of claim 1 with one or more ligands; and

(b) identifying a ligand that binds to one of
20 said variant nucleic acids.

US 5,557,741 A
11/23/97

SUB
A2
CO. 4.
II

(a) contacting the cell composition of claim 1 with one or more ligands, said cells containing a diverse population of variant polypeptides encoded by said variant nucleic acids; and

14. A cell composition comprising a population
10 of non-yeast eukaryotic cells containing a population of
10 or more variant nucleic acids, each of said variant
nucleic acids being expressed in a different cell and
integrated in the genome of each cell by a site specific
recombination sequence.

16. The cell composition of claim 14, wherein
20 said cells express Cre recombinase or Flp recombinase.

18. The cell composition of claim 17, wherein at least one of said lox sites is a loxP site.

20. The cell composition of claim 17, wherein
said site in the genome comprises two non-identical lox
5 sites.

22. The cell composition of claim 14, wherein
10 said variant nucleic acids are integrated at a single
site in the genome of each cell.

15 24. The cell composition of claim 14, wherein
said cell is a mammalian cell.

(a) screening the cell composition of claim 14
20 for an activity associated with a parent polypeptide of a
diverse population of variant polypeptides encoded by
said variant nucleic acids; and

(b) identifying a variant polypeptide exhibiting an optimized activity relative to said parent polypeptide.

26. A method of identifying a binding ligand, comprising:

(a) contacting the cell composition of claim 14 with one or more ligands; and

5 (b) identifying a ligand that binds to one of said variant nucleic acids.

27. A method of identifying a binding ligand, comprising:

(a) contacting the cell composition of claim 14 with one or more ligands, said cells containing a diverse population of variant polypeptides encoded by said variant nucleic acids; and

(b) identifying a ligand that binds to a polypeptide encoded by said variant nucleic acids.

28. A cell composition comprising a population of non-yeast eukaryotic cells containing a diverse population of 10 or more heterologous nucleic acid fragments, said heterologous nucleic acid fragments comprising distinct species of nucleic acid fragments, each of said heterologous nucleic acid fragments being expressed in a different cell and located within each cell at an identical site in the genome.

29. The cell composition of claim 28, wherein said heterologous nucleic acid fragments are integrated in each cell by a site specific recombination sequence.

31. The cell composition of claim 28, wherein said site in the genome comprises two lox sites.

33. The cell composition of claim 31, wherein at least one of said lox sites is a lox511 site.

35. The cell composition of claim 34, wherein said site in the genome comprises a loxP site and a lox511 site.

37. A method of identifying a binding ligand, comprising:

(b) identifying a ligand that binds to one of said heterologous nucleic acid fragments.

38. A method of identifying a binding ligand comprising:

- (a) contacting the cell composition of a cell line or more ligands, said cells containing a distribution of polypeptides encoded by said heterologous nucleic acid fragments; and
- (b) identifying a ligand that binds to a polypeptide encoded by said heterologous nucleic acid fragments.

39. A method of identifying a polypeptide or for a ligand, comprising:

- (a) contacting a population of non-yeast prokaryotic cells containing a diverse population of heterologous nucleic acid fragments encoding polypeptides with a ligand, said heterologous nucleic acid fragments comprising distinct species of nucleic acid fragments, each of said heterologous nucleic acid fragments being expressed in a different cell and located at each cell at an identical site in the genome;
- (b) identifying a polypeptide encoded by said heterologous nucleic acid fragments that binds to said ligand.

5

polypeptide encoded by said heterologous nucleic acid fragments.

10

15

20

40. A method of identifying a functional polypeptide fragment, comprising:

(a) introducing a diverse population of 10 or more heterologous nucleic acid fragments into a non-yeast eukaryotic cell to generate a population of cells, said heterologous nucleic acid fragments comprising distinct species of nucleic acid fragments, each of said nucleic acid fragments being expressed in a different cell and located within each cell at an identical site in the genome;

(b) screening said population of cells for a functional activity; and

(c) identifying a polypeptide encoded by said nucleic acid fragments having said functional activity.

SUB
A2
C2
657209-11004